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Relaxant effects of β-adrenergic agonists on porcine and human detrusor muscle

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Abstract

Aim: Relaxant effects of different β-adrenoceptor agonists on porcine and human detrusor were examined. Thus, the β-adrenoceptor subtype mainly responsible for relaxation in the detrusor muscle of pigs was characterized. Additionally, different effects of several β-agonists in both species were shown.

Methods: Experiments were performed on muscle strips of porcine and human detrusor suspended in a tissue bath. The relaxant effects of the non-selective β-agonist isoprenaline, the selective β2-agonists procaterol, salbutamol and the selective β3-agonists BRL 37344, CL 316 243 and CGP 12177 on potassium-induced contraction were investigated. The inhibitory effect of different substances on the maximum contraction and the rank order of potency for endogenous catecholamines was determined in pigs. Furthermore, concentration-relaxation curves were performed for pigs and humans.

Results: *Pigs*: In the pre-treatment experiments isoprenaline and procaterol showed similar effects. The concentration–response experiments showed that the maximum relaxation induced by procaterol and salbutamol was more than 90%, not significantly different from isoprenaline, whereas the maximum relaxations of CL 316 243, BRL 37344 and CGP 12177 amounted to 68, 70 or 30%, respectively. Rank order of potencies was isoprenaline ≥ adrenaline > noradrenaline. *Humans*: Isoprenaline, procaterol, salbutamol and CL 316 243 showed a maximum relaxation of 80, 41, 24 and 35% and pD2 values of 6.24, 5.65, 5.48 and 5.55, respectively.

Conclusion: β2-receptors play a main functional role in mediating relaxation of porcine detrusor. Selective β2- and β3-agonists similarly relax the human detrusor. Effects were smaller compared with the pig.

Keywords beta-adrenoceptor, beta-agonist, catecholamines, detrusor relaxation, human, organ bath, pig, smooth muscle.

Urinary bladder dysfunction is a widely spread disease. Older people and women are more often affected. One type of urinary incontinence is based on a hyperactive bladder or unstable bladder. In the USA, the overactive bladder affected 34 million individuals compared with 17 million with urinary incontinence during the year 2000 (Hu 2004). In an ageing population, the urinary incontinence is also a social and economic problem. In the USA, the total cost of urinary incontinence and overactive bladder for year 2000 was 19.5 billion dollars and 12.6 billion dollars, respectively (Hu 2004). At present, antimuscarinic drugs like oxybutynin, trospiumchloride and tolterodine are the therapeutic agents mainly used for detrusor instability (Andersson 2000a). Unfortunately, use is limited by different adverse effects generated by the antimuscarinic action like dry mouth, constipation and blurred vision (Yarker *et al.* 1995) and patients have to discontinue the

therapy. Therefore, drugs with other mechanisms of action are urgently needed. An interesting therapeutic approach is the use of β-adrenoceptor-agonists (Andersson 2000b). β-receptors play an important role in the relaxation of detrusor muscle via activation of adenylate cyclase. Both β2- and β3-receptors are suspected of mediating a major part of the relaxation via β-agonists. Studies investigating the detrusor muscle of pigs are rare, which β-adrenoceptor-subtype mainly mediates relaxation is not clarified. Since porcine detrusor muscle is suspected to have similar adrenoceptor (Goepel *et al.* 1997) and muscarinic receptor (Sellers *et al.* 2000) expression as human detrusor muscle, the detrusor muscle of pigs could serve as a model for the human one. In this study, the effect of the following β-adrenoceptor–agonists on potassium-induced (K^+: potassium) contraction of porcine urinary bladder smooth muscle were investigated: the non-selective β-agonist isoprenaline, the selective β2-adrenoceptor–agonists procaterol and salbutamol, the selective β3-adrenoceptor–agonists CL 316 243 and BRL 37344 and the selective β3-adrenoceptor agonist and β1- and β2-adrenoceptor antagonist CGP 12177 (Kaumann 1996). Furthermore, rank order of potency for endogenous catecholamines in porcine detrusor muscle was determined, since it is said to be characteristic for a special adrenoceptor subtype (Lands *et al.* 1967, Emorine *et al.* 1989). Additionally, selected β-agonists were examined on human detrusor muscle strips. By this way, our study allows the comparison between both species concerning the relaxant effects of different β-adrenoceptor–agonists. Even though the human tissue did not originate from patients with unstable bladder our results could provide useful information with regard to pharmacological treatment of unstable bladder, since it was shown by Restorick & Mundy (1989) that there was no difference in the density of β-adrenoceptors in hyperreflexic bladders compared with normal bladder.

Materials and methods

Porcine bladder

Urinary bladders of female pigs (breed: German Landrace) obtained from the abattoir in Mannheim were examined. After slaughtering, bladders were immediately removed and placed in Na^+ (sodium) Krebs solution. *N* represents the number of different animals, *n* represents the number of strips. The muscle strips were taken from the posterior wall of the bladder body.

Human bladder

Human bladder detrusor were obtained from 10 patients (eight men and two women). The average age was 67 ± 4.5 (mean ± SE). Patients underwent total cystectomy because of bladder carcinoma except for one woman who had open pelvic surgery inclusive cystectomy because of a carcinoma of the uterine cervix. All specimens were taken from macroscopically normal tissue of the posterior part of the bladder body, placed in Na^+ Krebs solution and gassed continuously with 95% O_2 and 5% CO_2. Experiments were performed on the day of the operation or 1 day later. For storage the tissue was placed in Na^+ Krebs solution, gassed continuously with 95% O_2 and 5% CO_2 for 1 h at room temperature and stored at 4 °C in Na^+ Krebs solution, pH 7.4. The experiments with human tissue were performed according to Helsinki declaration. Written informed consent was obtained from all patients before the operation was performed.

Preparation and equilibration

Smooth muscle strips approx. 8 × 3 mm were cut from the bladder body, mucosa and serosa were removed. Preparation was performed in Krebs solution. The tissues were mounted in 4 mL organ bath containing Na^+ Krebs solution which was maintained at 37 °C and gassed continuously with 95% O_2 and 5% CO_2. The preparation was gradually stretched until a stable tension of about 1 g was obtained. The tension of the strips was measured isometrically with force transducers (TIM-1020; FMI = Foehr Medical Instruments GmbH, Seeheim/Ober-Beerbach, Germany or TSE transducer; Technical and Scientific Equipment GmbH, Bad Homburg, Germany) and recorded using BEMON32 software (FMI) or TSE BIOSYS 2.1 software (TSE), respectively. Data were analysed using FMI VITRODAT 2.2A software.

Pre-treatment experiments

After the equilibration period of 1 h muscle strips were contracted with 124 mM K^+ solution. After maximum contraction had developed, preparations were washed four times until a steady resting level of tension was attained. A second contraction with 124 mM K^+ solution was performed after 30 min, then muscle strips were washed again four times. When a steady resting level was attained, muscle strips were equilibrated for 30 min with Krebs solution containing the appropriate concentration of the examined drug or vehicle as a time control. Concerning CL 316 243 the examined concentrations reached from 10^{-8} to 10^{-4} M, concerning procaterol and isoprenaline from 10^{-9} to 10^{-4} M. Each preparation was used for only one concentration or as a time control. After this equilibration period, the K^+-induced contraction was repeated.

Concentration–response curves

After equilibration the detrusor strips were pre-contracted with 30 mM K^+ solution. After the contraction had stabilized increasing concentrations of the β-adrenoceptor agonists were added cumulatively in 0.5 log unit increments to obtain concentration–relaxation curves. In this way, the concentration of the appropriate drug in the bathing fluid was increased from 10^{-10} to 10^{-4} M in the experiments with procaterol, salbutamol, adrenaline and noradrenaline, from 10^{-9} to 10^{-4} M concerning isoprenaline, CL 316 243, BRL 37344 and CGP 12177A. Each preparation was used for only one of the agonists. In the experiments with adrenaline and noradrenaline the alpha-adrenergic blocker phentolamine was present in a concentration of 10^{-6} M. After finishing the concentration–response curve of the selective β-agonists, isoprenaline in a concentration of 3×10^{-6} M was added to compare the maximum effect of the selective β-agonist with the maximum effect of isoprenaline. Once the tension did not change anymore, the tissues were washed four times with Krebs solution until a steady resting level was attained which was usually equal to or lower than that before evoking the contraction. In case of isoprenaline, tissues were directly washed after finishing the concentration–response curve. In order to recognize time-dependent changes in tension of the pre-contracted muscles, detrusor strips without pre-treatment served as time controls.

Solutions

The Na^+ Krebs solution (pH 7.4) had the following composition (mM): sodium chloride 119, potassium chloride 4.6, sodium bicarbonate 15, calcium chloride 1.5, magnesium chloride 1.2, sodium dihydrogene phosphate 1.2, glucose 1.98 g L^{-1}. The composition of the 124 K^+ solution, pH 7.4, was [mM]: potassium chloride 124, sodium bicarbonate 15, calcium chloride 1.5, magnesium chloride 1.2, sodium dihydrogene phosphate 1.2, glucose 1.98 g L^{-1}. The 30 mM K^+ solution was a mixture of the previously described solutions.

Drugs

The following drugs, purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany), were used:

(1) isoprenaline hydrochloride;
(2) procaterol;
(3) salbutamol;
(4) CL 316 243 (disodium 5-[(2R)-2-[[(2R)-2-(3-Chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate);
(5) BRL 37344 sodium salt, (±)-(R*,R*)-[4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]phenoxy]acetic acid sodium;
(6) (±)-CGP 12177 hydrochloride, 4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1, 3-dihydro-2H-benzimidazol-2-one hydrochloride;
(7) (R)-(−)-adrenaline;
(8) DL-noradrenaline hydrochloride;
(9) phentolamine hydrochloride.

Substances were dissolved as follows: isoprenaline in 30 mM K^+ solution for the concentration–response curve, when used as the reference drug in distilled water. CL 316 243 was dissolved in NaCl solution (0.9%), procaterol, salbutamol, BRL 37344, CGP 12177A and phentolamine in distilled water. Subsequent dilutions of the drugs were prepared in 30 mM K^+ solution, concerning procaterol in distilled water. The solutions were prepared on the day of the experiments.

Data

All results are expressed as mean values ± SEM.

Agonist potencies were expressed as a pD2 value, which is the negative logarithm of the EC50 value (≅ molar concentration of agonist resulting in 50% of the maximum response). Statistical analysis was performed using a Student's two-tailed *t*-test. A probability level of less than 0.05 was accepted as significant. Additionally, in case of the pre-treatment experiments the one-way analysis of variance (ANOVA) was performed.

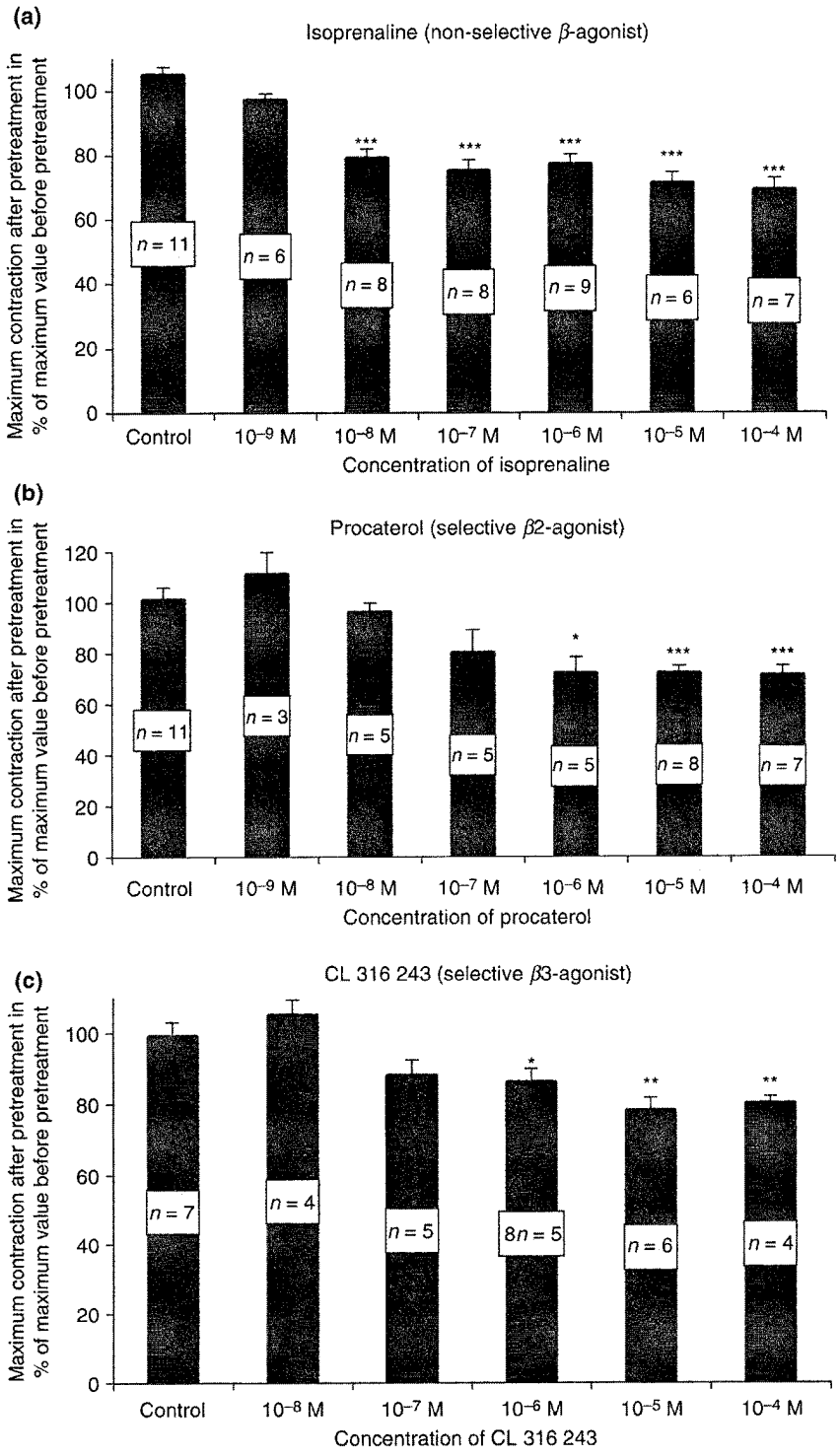
Results

Pre-treatment with isoprenaline, procaterol and CL 316 243 in the porcine detrusor muscle

Pre-treatment with isoprenaline (Fig. 1a) resulted in a significant reduction of the maximum contraction at concentrations from 10^{-8} to 10^{-4} M. The effect of procaterol (Fig. 1b) was similar to that of isoprenaline at higher concentrations. At lower concentrations the inhibitory effect of procaterol was not significant. The exact values are shown in Figure 1. Using the ANOVA approach the means within one group were also significantly different. The calculated pD2-values for isoprenaline, procaterol and CL 316 243 were 8.32, 7.35 and 7.12 respectively.

Concentration–relaxation curves of isoprenaline, procaterol and salbutamol in the porcine detrusor muscle

All drugs produced a concentration-dependent relaxation on K^+-pre-contracted smooth muscles. Maximum effects and mean pD2-values are shown in Table 1. The



pD2-value of procaterol was not significantly different from the pD2-value of isoprenaline. The addition of isoprenaline in a concentration of 3×10^{-6} M at the end of the concentration–relaxation curve of procaterol led to no further decrease (8.85% ± 1.99, $n = 28$, $N = 9$) This value was not significantly different from the maximum relaxation induced by procaterol. Concerning salbutamol, the addition of isoprenaline resulted in a decrease to 6.41% ± 0.81, not significantly different from the value obtained at the highest concentration of salbutamol. The mean cumulative concentration–relaxation curves are shown in Figure 2a.

Table 1 Porcine detrusor muscle: maximum effects (stated as tension to which the initial tension was decreased) and mean pD2-values of the examined substances are shown

Substance	Maximum effect (%)	Mean pD2-value
Isoprenaline	6.79 ± 1.01 (n = 20, N = 9)	8.29 ± 0.10 (n = 20, N = 9)
Procaterol	7.66 ± 1.95 (n = 28, N = 9)	8.00 ± 0.08 (n = 21, N = 7)
Salbutamol	7.50 ± 2.62 (n = 7, N = 5)	6.82 ± 0.17 (n = 7, N = 5)
CL 316 243	32 ± 3.5 (n = 16, N = 8)	6.28 ± 0.05 (n = 16, N = 8)
BRL 37344	29.72 ± 3.73 (n = 8, N = 6)	7.81 ± 0.15 (n = 8, N = 6)
CGP 12177	70.08 ± 6.33 (n = 9, N = 8)	7.02 ± 0.24, (n = 9, N = 8)
Adrenaline	6.96 ± 1.81 (n = 10, N = 5)	8.27 ± 0.35 (n = 10, N = 5)
Noradrenaline	20.43 ± 5.64 (n = 8, N = 7)	6.01 ± 0.12 (n = 8, N = 7)

N represents the number of different animals; and n represents the number of strips.

Concentration–relaxation curve of CL 316 243, BRL 37344 and CGP 12177 in the porcine detrusor muscle

The maximum effect of all β3-selective agonists was lower than that of the β2-agonists. Maximum effects and mean pD2-values are shown in Table 1. The pD2-values were significantly different from each other. After adding isoprenaline a further significant decrease to 10.15 ± 1.34% occurred in the concentration–relaxation curve of CL 316 243, concerning BRL 37344 the decrease to 25.75 ± 3.31% was not significantly different from that obtained at the highest concentration of BRL 37344. Concerning GGP 12177 the addition of isoprenaline led only to an insignificant decrease to 61.29 ± 7.85% whereas in controls without adding CGP 12177 final adding of isoprenaline led to a significant decrease to 12.26 ± 6%. Mean cumulative concentration–relaxation curves are shown in Figure 2b.

Rank order of potency for endogenous catecholamines determined in the porcine detrusor

Adrenaline induced relaxation with a higher potency than noradrenaline. Maximum effects and mean pD2-values are shown in Table 1. The addition of isoprenaline led to a decrease to 8.11 ± 2.55% in case of adrenaline and 12.36 ± 3.28% (n = 9, N = 8) in the case of noradrenaline, both not significantly different from the values induced by the catecholamines in the highest concentration. The pD2-value of adrenaline was significantly different of that of noradrenaline, but not significantly different compared with isoprenaline. The pD2-value of noradrenaline (6.01 ± 0.12, n = 8, N = 7) was significantly smaller compared with those of adrenaline and isoprenaline (P < 0.001). Hence, rank order of the potencies was determined as follows: isoprenaline ≥ adrenaline > noradrenaline. Mean cumulative concentration–relaxation curves to catecholamines and time control (n = N = 8) are shown in Fig. 2c.

Relaxant effects of isoprenaline, procaterol, salbutamol and CL 316 243 on the human detrusor

The mean concentration–relaxation curves of isoprenaline, procaterol, salbutamol and CL 316 243 are shown in Figure 3. Maximum effects and mean pD2-values are shown in Table 2. In case of procaterol the addition of isoprenaline led to a decrease to 32.33 ± 4.22%, significantly different (P < 0.001) from the value induced by procaterol at the highest concentration.

In case of salbutamol it caused a further decrease to 33.04 ± 4.62% which was significantly different (P < 0.001) from the value induced by salbutamol at the highest concentration. Concerning CL 316 243 the addition of isoprenaline led only to a further decrease to

Figure 1 Porcine detrusor: maximum contractions after pre-treatment for 30 min with isoprenaline (1a), procaterol (1b) and CL 316 243 (1c) in different concentrations. Mean values ± SE are shown. Maximum contraction was calculated as follows: the peak value of tension minus the baseline of tension before evoking the contraction. The mean of the maximum values of the first and second K^+-induced contraction (defined as 100%) was compared with the maximum contraction after pre-treatment (paired analysis). In addition, maximum contractions after pre-treatment were compared with the third contractions in the control group where only vehicle was added (unpaired analysis). Muscle strips of the same animal served as a time control, instead of the β-agonist vehicle was added. *Means P-value < 0.05, **stands for P-value < 0.01 and ***means P-value < 0.001 in Student's paired t-test. In control strips no significant difference was found. Compared with the third contraction of the untreated control group (Student's unpaired t-test) maximum contraction after pre-treatment with isoprenaline was significantly reduced at concentrations of 10^{-8}–10^{-4} M, after pre-treatment with procaterol it was significantly reduced at concentrations of 10^{-4}–10^{-7}. For CL 316 243 it was significantly reduced at concentrations of 10^{-4} and 10^{-5} M, for 10^{-6} M the determined P-value was 0.052. At lower concentrations as stated above no significant difference was found.

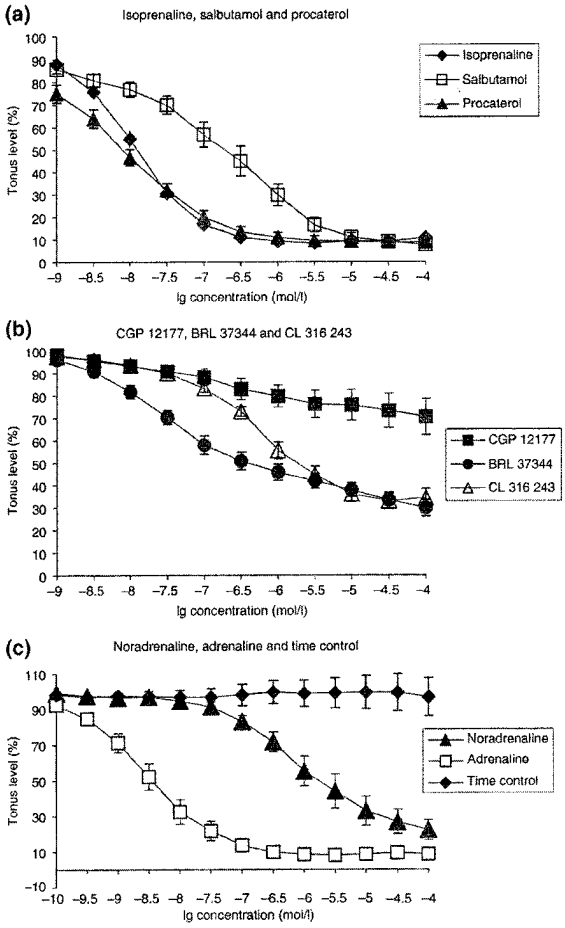


Figure 2 Porcine detrusor: mean cumulative concentration–relaxation curves: (a) the non-selective β-agonist isoprenaline ($n = 20$, $N = 9$) and the selective β2-agonist salbutamol ($n = 7$, $N = 5$) and procaterol ($n = 28$, $N = 9$); (b) the selective β3-agonists CL 316 243 ($n = 16$, $N = 8$) and BRL 37344 ($n = 8$, $N = 6$) and the selective β3-adrenoceptor agonist and β1- and β2-adrenoceptor antagonist CGP 12177 ($n = 9$, $N = 8$); (c) the endogenous catecholamines adrenaline ($n = 10$, $N = 5$) and noradrenaline ($n = 8$–11, $N = 9$) and appropriate time control ($N = n = 8$). Vertical lines indicate SEM. Experiments were performed after pre-contraction with 30 mM K^+ solution. The reduced tension after adding each concentration is expressed as a percentage of the maximal value which was defined as the difference between the stable tension after adding the K^+ solution and the steady resting tension at the end of the experiments after several times of washing.

50.20 ± 4.19%, significantly different ($P < 0.001$) from the value induced by CL 316 243 at a concentration of 10^{-4} M. There was no significant difference between the pD2 values of all substances except for the pD2 value of CL 316 243 which was significantly different ($P < 0.01$) from the value of isoprenaline. In control strips where only vehicle was added ($n = 22$, $N = 8$) no significant changes in tension occurred.

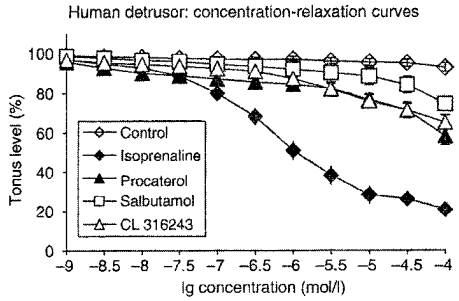


Figure 3 Human detrusor: mean cumulative concentration–relaxation curves of the non-selective β-agonist isoprenaline ($n = 11$, $N = 7$), the selective β2-agonists procaterol ($n = 10$, $N = 7$) and salbutamol ($n = 7$, $N = 6$) and of the selective β3-agonist CL 316 243 ($n = 12$, $N = 8$). Vertical lines indicate SEM. Experiments were performed after pre-contraction with 30 mM K^+ solution. The reduced tension after adding each concentration is expressed as a percentage of the maximal value which was defined as a value between the pre-contracted level immediately before adding the first concentration and the steady resting level at the end of the experiment after washing several times.

Discussion

In this study, the relaxant effects of different β-adrenoceptor agonists on the detrusor muscle of pigs and on human detrusor were examined. In two types of experiments we used K^+ induced contractions. The reason was that when performing concentration–relaxation curves the application of the examined substance is repeated several times on the same muscle strip. Since there is the possibility that the effect of the agent is weaker at the end of the experiment, we decided to perform an additional type of experiment in which each muscle strip was used for only one concentration of a substance. By this way, in the described pre-treatment experiments any decrement of the pharmacological effect which could be caused by repeated application of the agent was avoided. Furthermore, high potassium solution was chosen because several contractions of the same muscle strip induced by potassium solution were very stable. For pre-contraction we did not use a physiological agonist like carbachol or acetylcholine because it was found that isoproterenol was less potent and efficacious at relaxing carbachol–pre-contracted rat bladder strips than KCl–pre-contracted strips (Longhurst & Levendusky 1999). One possible reason is that carbachol caused a functional antagonism of the isoproterenol-induced relaxation by inhibiting adenylate cyclase through its interactions with M2-receptors and thus decreasing cyclic AMP concentrations. Longhurst & Uvelius (2001) suggest that agents used to precontract smooth muscle strips should be carefully selected to ensure that the second messenger systems

Table 2 Human detrusor muscle: maximum effects (stated as tension to which the initial tension was decreased) and mean pD2-values of the examined substances are shown

Substance	Maximum effect (%)	Mean pD2-value
Isoprenaline	20.25 ± 3.59 (n = 10, N = 7)	6.24 ± 0.12 (n = 10, N = 7)
Procaterol	59.15 ± 4.01 (n = 10, N = 7)	5.65 ± 0.34 (n = 10, N = 7)
Salbutamol	75.91 ± 31.38 (n = 7, N = 6)	5.48 ± 0.45 (n = 7, N = 6)
CL 316 243	65.26 ± 3.71 (n = 12, N = 8)	5.55 ± 0.18 (n = 12, N = 8)

N represents the number of different human beings; and n represents the number of strips.

they activate or inhibit do not modulate responses to the relaxing agent.

In porcine detrusor muscle the maximum relaxation induced by the β2-agonists procaterol and salbutamol was more than 90% which was not significantly different from that obtained when using isoprenaline. Maximum relaxation induced by the selective β3-agonists was smaller. The pD2-values of isoprenaline and procaterol did not differ significantly showing that procaterol is as potent as isoprenaline. In addition, the rank order of the relaxing potencies for catecholamines on pre-contracted porcine detrusor muscle was determined as follows: isoprenaline ≥ adrenaline > noradrenaline. For β2-adrenoceptors it is said to be (Lands *et al.* 1967): isoprenaline > adrenaline > noradrenaline, for the β1- and β3-subtype it is: isoprenaline > noradrenaline > adrenaline (Lands *et al.* 1967, Emorine *et al.* 1989). Therefore, the rank order determined in this study pointed very clearly to the β2-receptor subtype as main subtype. Studies investigating the detrusor muscle of pigs are rare, the β2-receptor subtype as well as the β3-subtype was postulated as the one which mainly mediates relaxation. Goepel *et al.* (1997) showed that β2-adrenoceptors predominate in porcine and human detrusor using competition binding experiments with the β2-selective receptor antagonist ICI 118551 and the β1-selective receptor antagonist CGP 20712A. Yamanishi *et al.* (2002) showed that the relaxation of porcine detrusor muscle is mediated via both the β2- and β3-adrenoceptor-subtypes. In their functional studies isoprenaline and the β2-selective agonist salbutamol relaxed KCl-pre-contracted muscle strips with high potency (pD2-value 7.7 and 7.2, respectively), whilst CGP 12177 and BRL 37344 had a lower potency and were partial agonists with pD2-values of 4 and 6.9, respectively. In their study, salbutamol showed a mean maximum relaxation of 86% of that obtained to 30 μM isoprenaline, whereas CGP 12177 and BRL 37344 showed a mean maximum relaxation of 40 and 59%, respectively. In our study, CGP 12177 also led to the smallest mean maximum relaxation. So the functional studies of Yamanishi *et al.* (2002) showed that the β2-agonist is more potent than the β3-agonists in porcine detrusor muscle. These functional results are similar to our own data. Even though, based on competition binding experiments, the authors suggest that the β3-adrenoceptor is the predominant population in detrusor muscle of pigs. Larsen (1979) showed in isolated carbachol-contracted bladder strips of porcine detrusor muscle that the relaxing potency of isoprenaline was four times that of salbutamol and 30 times that of noradrenaline. The maximum relaxant effect was 100% with both isoprenaline and salbutamol. Procaterol was not examined. These results are similar to our own data where salbutamol showed a maximum relaxant effect, not significantly different from that of isoprenaline, but had a lower pD2 value. In our study, noradrenaline was also much less effective than isoprenaline and adrenaline.

Furthermore, our results also indicate that the β-receptor-subtype mediating relaxation of detrusor muscle is species dependent. Between porcine and human detrusor the contribution of the β2- and β3-subtype seems to be different. It is known that in some species several different subtypes contribute to relaxation. In rabbits the β2-receptor was shown to be mainly responsible for relaxation (Yamazaki *et al.* 1998), in cynomolgus monkey detrusor it was the β3-subtype (Takeda *et al.* 2002). Takeda *et al.* (2003) found in canine detrusor muscle that relaxing potency of CL 316 243 was bigger than that of procaterol indicating a pronounced role of β3-adrenoceptors in dogs. Longhurst & Levendusky (1999) suggest that rat urinary bladder body contains β1-, β2- and β3-receptors, all of which mediate relaxation, whereas Yamazaki *et al.* (1998) suggest that both β2- and β3-receptors mediate relaxation in rats.

In our study, isoprenaline is 100-fold less potent on human than porcine detrusor muscle. This result suggests a much smaller receptor population in human tissue and underlines differences between the two examined species concerning the receptor densities. Interestingly, organ bath experiments on human detrusor muscle point to the β3-adrenoceptor subtype being the main one in the human bladder (Igawa *et al.* 1999, Takeda *et al.* 1999). Igawa *et al.* (2001) found in human detrusor preparations from patients with a normal bladder that the pD2 values for isoprenaline,

CL 316 243 and GCP 12177 were: 6.36, 5.53 and 5.53, respectively. In their study, the mean maximum relaxation of the two above-mentioned β3-agonists was about 50% of the isoprenaline-induced one. In our study, the determined pD2 values of isoprenaline and CL 316 243 were similar: 6.24 and 5.55, respectively, and CL 316 243 induced only 35% maximum relaxation, whereas isoprenaline induced 80% relaxation. Hence, concerning isoprenaline and the β3-agonist, results are similar. Igawa *et al.* examined only one substance belonging to the β2-agonist: procaterol. But in contrast to our results, it showed no relaxation up to 10^{-5} M. Authors suggest that in humans a major portion of the relaxant effect of isoprenaline is mediated via β3-adrenoceptor stimulation. Our study indicates that both β2- and β3-agonists relax human detrusor, concluding that both receptor subtypes are responsible for relaxation. This supposition is underlined by the clinical trials of Grueneberger (1984) and Grueneberger & Geier (1981). Clenbuterol, another β2-specific adrenergic agent, was successfully used to treat 20 women with motor urge incontinence (Grueneberger 1984). Ten women reported complete relief of symptoms and urge incontinence was reduced in five. The number of side effects was small. This implies that β-adrenergic agonists could be a good new therapy for bladder overactivity, possibly in combination with anticholinergic drugs.

Our results indicate that the β2-adrenoceptor-subtype appears to be the most functionally important β-adrenoceptor in the pig bladder while both the β2- and β3-adrenoceptors appear to be equally important in mediating human detrusor smooth muscle relaxation. Effects on human detrusor muscle were smaller compared with the pig.

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